Lasers in Surgery and Medicine 18:391–396 (1996)

Copper Vapor Laser and Photocarcinogenesis in Hairless Mice

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Background and Objective: **Port wine stains are commonly located on UV-exposed skin areas. We therefore examined the long-term interaction between UV radiation and copper vapor laser light (578 nm, yellow light) and whether the thermal influence from laser light had a carcinogenic potential itself.**
Study Design/Materials and Methods: **The study was conducted in lightly pigmented hairless hr/hr C3H/Tif mice and included 8 groups of 17–20 mice. Intensities of 0.5, 1.0, and 1.4 W were used, corresponding to calibrated Hexascan fluences of 15.9, 31.8, and 44.6 J/cm^2. Beam diameter was 1 mm and pulse duration 250 msec. UV irradiation of the mice was performed 4 days weekly and started the day after laser treatment. The UV simulated solar ultraviolet radiation came from one Phillips TL 12 and five Bellarium-S SA-1-12 tubes. The daily dose was 1.3 J/cm^2, equivalent to 2.1 B-MED.**
Results: **No tumors appeared in groups receiving laser light only. The time to first ($P < 0.01$), second ($P < 0.01$), and third ($P < 0.02$) tumor was significantly delayed in the group treated with 1.4 W before UV irradiation ($P < 0.01$) compared with those receiving UV radiation only. No significant differences could be demonstrated for the groups treated with 0.5 and 1.0 W.**
Conclusion: **One laser treatment with the copper vapor laser did not accelerate UV-induced photocarcinogenesis, and the laser exposure did not have a malignant potential itself.**

Key words: laser surgery, adverse effects, skin radiation, skin neoplasms

INTRODUCTION

Medical lasers with wavelengths corresponding to or close to 577 nm, which is one of the absorption peaks of hemoglobin, are mainly used in the treatment of cutaneous vascular malformations, of which port wine stains constitute a great part because of their abnormal cosmetic appearance [1,2]. The principles of laser therapy are to induce specific vascular damage and fibrosis with sparing of overlying skin and skin appendages. The aim is to induce a minimum of nonspecific damage due to unwanted absorption by melanin and due to heat diffusion and scatter from the main absorbing chromophores, oxyhemoglobin, and melanin, thereby inducing a second degree burn [3,4].

Port wine stains are commonly located on the face, and therefore have the possibility of being exposed to sunlight [5]. However, the interaction between laser light and UV radiation has only been described partially: A high degree of epidermal skin pigmentation, which may be induced by UV irradiation, reduces the vascular specificity of laser exposure [6,7] and is responsible for an outcome with a higher degree of side effects [8–10]. Moreover, we have previously reported laser-induced wound healing and scarring to be influenced by UV exposure [11], whereas malignancy following laser and sun exposure, to our knowledge, has not been investigated. How-

Accepted for publication March 20, 1995.

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TABLE 1. Treatment Schedule for Eight Groups of Mice, Receiving Different Intensities of Laser Light (W/spot) With or Without Subsequent UV Irradiation

Group	No. mice	Laser intensity (W)	UV irradiation
1	20	0	−
2	20	0	+
3	20	0.5	−
4	19	0.5	+
5	18	1.0	−
6	20	1.0	+
7	19	1.4	−
8	17	1.4	+

ever, dermatological laser treatment has so far been considered safe, since the action spectrum of lasers in the visible range lies outside the known range of induction of erythema, pigmentation, and photocarcinogenesis [12,13].

Thermal burns have been described to possess the potential for malignant transformation, although this seldomly occurs [14–16], and a case has been reported that describes abnormal epidermal changes similar to those of actinic keratosis after argon laser therapy [17]. We therefore examined whether laser light has a carcinogenic potential itself and whether laser exposure prior to UV irradiation accelerates the carcinogenic potential of UV radiation, which serves as a complete carcinogen, since both tumor initiation and promotion are induced by UV light [18].

MATERIALS AND METHODS

Animals

Lightly pigmented, hairless, female hr/hr mice with C3H/Tif background (n = 153, Bomholt Breeding and Research Center, Denmark) were randomly distributed into 8 groups containing 17–20 mice each (Table 1). The animals were 14–15 weeks old at the start of the experiment and were weighed regularly. The mean weight ± SEM was 23 ± 0.2 g at the start of the experiment. The animals had free access to water and standard laboratory chow (3,100 kcal/kg) and were kept on a 12-hr light/dark cycle. The room temperature was kept between 23°C and 24°C. The animals were irradiated in their boxes from above, and they were free to move during irradiation. The mice were killed when necessitated by tumor development, that is, when the total tumor burden was ≥ 3 cm^2, by age, or at the end of the observation period.

Laser Techniques

The CVL, Multilase, (PBI Medical, Denmark) was used in connection with a microprocessor-controlled handpiece, a Hexascan device (Prein & Partners, Ferney-Voltaire, France). The laser pulses were applied to the skin from a fixed distance and in a fixed jumping mode with the purpose of minimizing thermal diffusion and obtaining a high degree of uniformity of the treated areas. The Hexascan has an indicator accuracy of ±0.05 watts. The CVL operates in a quasi-continuous mode, producing a rapid train of pulses and emitting 8,400 pulses/sec (8.4 kHz) at the 578 nm, yellow band. The beam diameter was 1 mm and the pulse duration was 250 msec. Intensities of 0.5, 1.0, and 1.4 W/spot were used, corresponding to calibrated Hexascan fluences of 15.9, 31.8, and 44.6 J/cm^2. Laser intensities were controlled by a power meter (Analogue Power Read Out no. 25 APR, Power Meter Head no. 25 V-VIS, Photon Control).

UV Radiation Sources

Simulated solar UV radiation was obtained from a bank of tubes consisting of one Phillips TL 12 and five Bellarium-S SA-1-12 tubes. The emission spectrum is shown in Figure 1. The emission spectrum of the radiation source was measured in 1 nm steps using a Jobin Yvon monochromator H10 double UV (slit widths 0.5, 1.0, and 0.5 mm) with a selective mirror blocking out visible light, and an International Light (IL) 1700 research radiometer with a SHD 033 detector. The monochromator was calibrated with an Optronic Laboratories deuterium lamp precision source, model 45D. The intensity of the UV source was measured with the IL 1700 research radiometer with an IL SED 400 detector, a WBS 320 filter, and a quartz diffuser. Measurements were performed under the grid of the cage at mouse back level. The intensities were corrected on basis of the spectral sensitivities of the detectors and the emission spectrum of the source. Exposure doses in Basic-MEDs (B-MED) were derived from the erythema effectiveness spectrum, the CIE erythema action spectrum of McKinlay and Diffey [19], and taking as a reference for one B-MED a 24-hour MED at 296 nm of 31.2 mJ/cm^2 [13]. The daily dose of 1.3 J/cm^2 is equivalent to 2.1 B-MED.

Experimental Design

The laser treatment was performed on the first day of the trial. Four equally sized areas

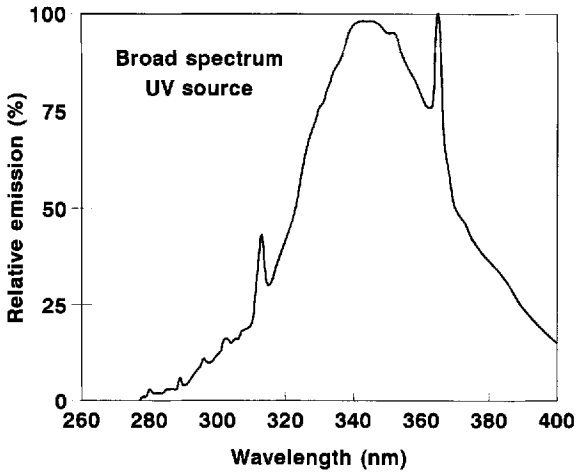

Fig. 1. Relative spectral emission of the simulated solar UV source.

(1.26 cm^2) on the dorsal skin of the mice were exposed to identical laser doses. Prior to laser treatment the mice were anaesthetized by intraperitoneal injections of fluanisone, fentanyl, and midazolam, and the mice were stretched in order to smooth out the skin and to plane the dorsum. Two spots were tattooed on the back, functioning as fix points for the laser application. It was thus possible to determine whether tumors developed inside or outside the previously laser treated areas. During laser therapy the room temperature was kept between 24°C and 26°C. UV irradiation started the day after the laser exposure and was performed four times weekly during the entire experimental period of 18 months, with each irradiation period lasting 8 minutes.

Registration and Statistics

The first three tumors with a diameter of ≥1 mm were mapped separately for each animal. Tumors were only included for statistical evaluation when they developed in previously laser-treated areas or in an adjoining border zone of 2 mm. This made us operate with a test area of approximately 6 cm^2, which covers the central, prominent part of the mouse back. A corresponding reference area was chosen on the UV irradiated control group, and tumors appearing outside this area were excluded. Tumor size was noted weekly and the time of death was registered.

Statistical analyses included the Mann-Whitney test and the Kaplan-Meier method, which calculated and visualized the probability of survival without a tumor in relation to the duration of the experiment in weeks. The time from the start of the experiment to the appearance of the first, second, and third tumor with a diameter ≥1 mm was analyzed using the log-rank test [20].

Pathology

In our previous tumor studies histological examinations were performed on all mice. These studies demonstrated that all larger tumors were squamous cell carcinomas (SCC). In this study the solar UV-irradiated mice were killed when required due to tumor development. This allowed the tumors to grow excessively. Therefore no histological examination was performed since all tumors were obviously malignant.

RESULTS

Only UV-irradiated mice developed tumors. No tumors appeared in the untreated or laser-treated groups within the 18 months of observation. The laser intensities used induced a broad spectrum of chronic skin reactions, the severity of which increased with the use of increasing laser intensities. Skin reactions ranged from no visible changes, a just visible texture change, or a slight degree of hyperpigmentation in the groups treated with 0.5 W, to clear hyperpigmentation and atrophic scarring with a variable degree of constriction in the groups treated with higher laser intensities. UV irradiation after laser exposure tended to accentuate laser-induced hyperpigmentation, to induce a border zone of palpable infiltration in the atrophic scars, and to quicken the wound healing process in the 1.4 W + UV–treated group as compared with the only 1.4 W-treated group (median values 24 days vs. 27 days). However, the difference was not significant.

The Kaplan-Meier plot in Figure 2 shows the probability of survival without a first tumor for those groups developing tumors. The time to 50% of the mice having their first tumor was 23 weeks for both the UV-irradiated and for the UV-irradiated + 0.5 W laser–treated group; 24 weeks for the UV-irradiated + 1.0 W laser–treated group, and 25 weeks for the UV-irradiated + 1.4 W laser-treated group. For the simulated solar UV + 1.4 W laser-treated group, the time to appearance of first ($P < 0.01$), second ($P < 0.01$), and third ($P < 0.02$) tumors was significantly delayed as compared with the group receiving solar UV only. No significant differences could be demonstrated for the groups treated with UV + 0.5 W and UV + 1.0 W laser exposure when comparing the time

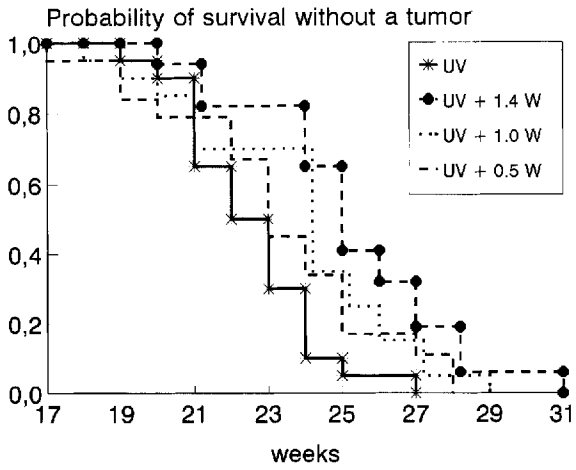

Fig. 2. Kaplan-Meier plot showing the time to first tumor for the groups exposed to 0.5 W, 1.0 W, and 1.4 W laser intensities prior to UV irradiation.

from the beginning of the experiment to the onset of the first, second, or third tumors.

Tumors developed in previously laser-treated areas and in adjoining nonexposed border zones, both of which are located at the most prominent place of the mouse back, corresponding to the area that received the highest dose of UV radiation. For registered tumors it was noted whether they appeared in the laser-treated areas, which macroscopically varied from uninvolved skin to atrophic, constricted skin, or whether they appeared in the adjoining nonexposed border zone. It was found that significantly less tumors ($P < 0.05$) developed in the atrophic skin areas, which were pretreated with 1.4 W before UV irradiation (83% of registered tumors), than in the comparable only UV-irradiated skin areas (93% of registered tumors). No significant differences were demonstrated in tumor appearance in the laser-treated areas for those groups treated with solely UV irradiation (93%) and those pretreated with 0.5 W (92%) and 1.0 W (95%).

DISCUSSION

Our study showed that the time from the beginning of the experiment to the appearance of tumors was prolonged when pretreatment with increasing laser intensities was performed. The 1.4 W + UV–treated mice developed during the observation period of 18 months (the mean lifetime for hairless mice is approximately 2 years), a significantly reduced degree of tumors in previously laser treated areas, (83% of registered tumors) in comparison with the corresponding UV irradiated but not laser-treated reference areas (93% of registered tumors). This means that a relatively higher degree of tumors developed in the border zone of the 1.4 W + UV–treated group in contrast to the reference border zone of the only UV-irradiated mice. This made us propose that the prolonged time to tumor formation might be caused by the time necessary to form a new epidermal layer. The oncogenic influence from UV radiation may thus first take place when new epidermal cells are formed. Another explanation for retarded tumor development may be that laser induced escars, may have blocked the UV penetration (median values of the wound healing time varied from 7 days in the 0.5 W + UV–treated group to 24 days in the group treated with 1.4 W + UV irradiation).

Various angles of approach have been chosen when dealing with the question of whether visible light is carcinogenic. For instance, the molecular effects of visible light have been examined, and Peak et al. have reported that it is probable that visible light is not mutagenic, although there is the reservation that experimental fluences are below levels that kill cells during realistic exposure periods [21]. Furthermore, it is generally assumed that no carcinogenicity appears in the visible range of the photobiological spectrum [12]. However, animal experiments to obtain direct information on carcinogenesis from visible light have been sparse. Griffin et al. studied the effect of visible light on UV-induced carcinogenicity, and their results indicated an activation of UV-induced ear tumors by white light [22]. However, no statistical calculations were performed and a fluorescent lamp with a slight UV emission at wavelengths shorter than 330 nm was used to emit white light. This UV emission might have accounted for the increased carcinogenesis.

Apfelberg et al. examined malignant transformations of fibroblasts, which in vitro had been exposed to argon and CO_2 laser [23]. They demonstrated that neither argon laser (488 nm and 514 nm) nor CO_2 laser (10,600 nm) induced a significant degree of malignant transformations, and they concluded that long-term clinical use of the two lasers could be considered safe. However, a case has been reported that describes the development of abnormal epidermal changes similar to those of actinic keratosis with disorganized cell layers and marked cytologic abnormalities 10 months after argon laser treatment of a 26-year-old woman [17]. This is, to our knowledge, the

only case reported to develop premalignant epidermal changes after argon laser therapy. However, only a few studies have extended their observation periods to be able to examine long-term epidermal changes [24–26], and no reports of carcinogenesis have been given.

Unspecific damage prior to UV radiation has been described by Urbach et al. to accelerate the appearance of UV-induced skin cancer, and the hypothesis was proposed that UV-induced skin cancer might be related to epidermal hyperplasia secondary to severe nonspecific skin damage [27]. However, the results obtained in our study could not confirm epidermal cell proliferation secondary to thermal damage to accelerate the rate of UV-induced tumor formation. Neither could we in our murine model confirm thermal injuries to have a malignant potential, although the association between thermal burn scars and neoplasia has been recognized for many years [14–16].

We designed our study to have just one laser treatment prior to UV irradiation, although it would have been more comparable to a clinical treatment program if the skin had been exposed to laser light more than once. However, our study is the first in vivo examination of the long-term carcinogenic risk of using 578 nm laser light, and on this basis we concluded that one treatment with the copper vapor laser does not have a malignant potential itself, just as we concluded that UV-induced photocarcinogenesis is not accelerated when a single laser exposure is performed prior to UV irradiation.

ACKNOWLEDGMENTS

This study was supported by Danish Cancer Society grant 90-2403. Technical assistance was provided by PBI Medical, Denmark and Benedicte Wulf.

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